

AUTONOMOUS SUPPORT FOR MICROORGANISM RESEARCH IN SPACE

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Abstract

A preliminary design for performing on-orbit, autonomous research on microorganisms and cultured cells/tissues is presented. An understanding of gravity and its effects on cells is crucial for space exploration as well as for terrestrial applications. The payload is designed to be compatible with the COMercial Experiment Transported (COMET) launch vehicle, an orbiter middeck locker interface, and with Space Station Freedom. Uplink/downlink capabilities and sample return through controlled reentry are available for all carriers. Autonomous testing activities are preprogrammed with inflight reprogrammability. Sensors for monitoring temperature, pH, light, gravity levels, vibration, and radiation are provided for environmental regulation and experimental data collection. Additional experimental data acquisition includes optical density measurement, microscopy, video, and file photography. Onboard full data storage capabilities are provided. A fluid transfer mechanism is utilized for inoculation, sampling, and nutrient replenishment of experiment cultures. In addition to payload design, representative experiments were developed to ensure scientific objectives remained compatible with hardware capabilities. The project is defined to provide biological data pertinent to extended duration crewed space flight including crew health issues and development of a Controlled Ecological Life Support System (CELSS). In addition, opportunities are opened for investigations leading to commercial applications of space, such as pharmaceutical development, modeling of terrestrial diseases, and material processing.

Introduction

Gravity is easily taken for granted, but its constant inertial acceleration affects every aspect of our lives. In fact, gravity affects all Earth's life forms and has done so throughout evolution. The fight against gravity has led to the formation of extremely strong biological support structures such as cellulose, chitin, and bone. Animal movement must first counteract the force of gravity, therefore, muscle and other methods of movement (flagella, cilia, and contractile filaments) must reflect this in their structure and function. Gravity is also responsible for processes such as convection and sedimentation that cells and organisms have evolved to use. Life on Earth today is highly diverse and constantly changing, but no matter what the organism or its habitat, gravity has surely played an important role in its development and life cycle.

Many biological experiments have been performed in the microgravity environment of space to determine what influence gravity has on life. The results: gravity does play an important role in the development and maintenance of life, but the specific mechanisms of gravity perception, adaptation, and use are not well understood. For example, the bodies of astronauts are dramatically altered in microgravity. Bone and muscles decrease, the immune system is weakened, and cardiovascular and neurovestibular systems that control circulation and balance change. Major adaptations adjust the body to the new reduced gravity environment. But how is the presence or absence of gravity sensed by a bone or muscle cell? Why do cells and organisms respond to gravity the way they do? How can these gravitational responses be inhibited to insure astronaut health or enhanced to produce new plants or microorganisms with special desirable traits? The answers to these and many similar questions are unclear, and they will remain unclear until

biology and microbiology can be studied easily and extensively in microgravity.

Space Habitation, a NASA/USRA (National Aeronautics and Space Administration/Universities Space Research Association)-sponsored advanced design class at the University of Colorado, is devoted to addressing issues concerning space life sciences and the commercialization of outer space. In an effort to make the microgravity environment of space more easily accessible for biological research and commercial application, the Spring 1992 class has developed a design for a small, versatile, biological research tool called the Cell Module for Autonomous Space Support (C-MASS). C-MASS meets many current needs for biological research in space and is responsive to the changing directives of today's U.S. space program which emphasize reliable, faster, better, and less expensive missions.

Background

Since 1958, the U.S. space program has brought the mysteries, challenges, and achievements of space exploration home to America. Recently, millions of viewers witnessed three space-walking astronauts from the Space Shuttle Endeavour working together to capture a stranded communications satellite by hand when hardware built for the job failed to work. The excitement and intrigue generated by space activities such as this provide an incentive propelling the nation forward in science and technology. However, even more important gains have come from scientific information and the many spin-off products and technologies derived throughout the space program. Space exploration, transportation, and life support challenge the limits of today's technology. Advancements in automation, computer technology, miniaturization, and remote sensing have followed. Spin-offs from those advancements include insulative and fire retardant materials, recycling technology, computer software, imaging systems, and medical techniques. Spin-offs mean better products, an increased standard of living, and consumer savings. For example, biotelemetry (the remote sensing of blood pressure, heart rate and rhythm, and temperature using very small, durable, light-weight sensors) was originally developed as a ground-based method to monitor astronauts. Now, biotelemetry packages are used to safely monitor heart attack patients in their own homes. This allows them to return to their

normal activities and eliminates the need for prolonged hospitalization and related medical costs.¹ Excitement in the space program is generated by human achievements like the satellite capture and the economic/technical importance of the space program arises from spin-offs that touch the lives of millions of people each and every day.

An area of great potential is space life sciences; this discipline addresses the issues, among others, of Controlled Ecological Life Support Systems (CELSS), astronaut health, and basic gravitational biology. The bioregenerative aspects of CELSS will greatly reduce the costs and Earth-dependency of life support systems, providing a means to fulfill long-term NASA goals such as a permanent return to the moon. Due to the lack of gravity in orbit, astronauts suffer from accelerated forms of many common ailments found on earth including osteoporosis (mineral loss in bones), muscle atrophy, space sickness, and cardiovascular alterations. For example, on Earth osteoporosis affects over 24 million elderly American today and is the cause of 1.3 million fractured bones each year (at an annual consumer cost of \$7-10 billion).² Developing treatment for the health problems astronauts face will lead to cures for diseases people suffer on Earth, saving lives, productivity, and money.

Basic gravitational biology focuses on the basic effects of gravity and mechanisms of gravity sensing in cells. With an understanding of the effects and perceptions of gravity at the level of the single cell, scientists may find ways to use the unique microgravity environment of space to perform biological manipulations or processes not possible on Earth. The value of these biological experiments could be well worth the high investment required for development and flight time. For instance, a single cell genetically altered in space to produce a beneficial byproduct could be brought back to Earth to reproduce, creating entire populations of cells or organisms with the same beneficial trait. In addition, the combination of gravitational biology and the unique environment of space may open the door to future commercial development of space.

The goals of space life sciences and the returns it will provide cannot be achieved instantly. A phased mission approach is required which employs many small missions, each contributing new technology, information, protocols,

and even spin-offs to bring the space program closer to its long-term goals in a step-by-step process. The phased mission approach is consistent with the Space Exploration Initiative (SEI), the new directive for the U.S. space program. SEI has a twofold strategy: "First, to develop and conduct small scale robotic/automated precursor missions designed to fill gaps in the nation's scientific and technological knowledge," and second to establish a "management culture" that can be relied upon to get the job done on time and for less money.³ This new emphasis on smaller, low-cost payloads will allow industries and research organizations to get involved, transforming the space program into a search for commercial applications and developments as well as a mission of science and exploration.

Space life sciences is one of the gaps in scientific and technological knowledge to which SEI refers. As an empirical science, it depends on multiple tests done in the space environment. Early missions placed little emphasis on life sciences beyond the minimum necessary to sustain humans for a voyage to the moon and back. Today, principal investigators like biologists and physicians who are not directly involved with NASA need greater access to space.

Unfortunately, there is no way to learn how gravity is sensed or what the extent of its effects on life are without performing experiments in the microgravity environment of space. The costs of sending even small packages into orbit are extremely high, and stringent NASA requirements make flight-qualified hardware complex and time consuming to develop. Machines on Earth such as the clinostat (which slowly rotates specimens to produce a constantly reorienting gravity vector that averages over time to zero) and the centrifuge can only be used to alter how gravity is perceived by organisms. Short-term microgravity environments achieved on KC-135 aircraft or sounding rockets are only somewhat helpful because they do not produce long enough periods of microgravity for many biological experiments.

To fill the gap in scientific and technological knowledge for space life sciences, an effective infrastructure for biological experimental hardware must be in place. This will make more frequent and longer duration experiments possible using new generic hardware with variable capabilities to cut through integration costs and NASA paperwork difficulties.

Rationale/Overview

The role of space habitation has been to address space life science issues and support the further exploration and commercialization of space through design work. In the past the class has focused on missions that would generate interest and excitement for the U. S. Space Program.

These projects concentrated on developing a CELSS and achieving the NASA long-term goal of returning to the moon. However, to accommodate the Space Exploration Initiative and immediate problems facing space life scientists, Space Habitation has recently turned its focus toward smaller missions emphasizing basic biological science and potential commercial applications. The design response developed by the Spring 1992 semester class is called the Cell Module for Autonomous Space Support (C-MASS).

C-MASS is a small autonomous payload designed to support on-orbit testing for a variety of microorganisms and cultured cells/tissues for periods of up to 30 days. It uses only existing or modified off-the-shelf hardware and currently available technology, thereby minimizing cost and maximizing reliability. C-MASS is designed for many types of experiments. It brings together an extensive variety of data acquisition capabilities not integrated in any existing space hardware of its size. The large commitment to data acquisition provides a means to obtain detailed information inflight instead of having to rely solely on the analysis of returned samples that cannot reveal time-dependent gravitational effects. C-MASS is also designed for compatibility with the Shuttle middeck locker, SpaceHab, Spacelab, COMercial Experiment Transporter (COMET), and Space Station Freedom (SSF). Carrier Versatility enables C-MASS to take advantage of benefits offered by each: access to frequent Shuttle missions, 30-day missions, and very low gravity levels on COMET, and even longer missions as well as extremely low gravity levels on the initial crew-tended stages of SSF. To perform onboard control experiments, a 1-g centrifuge is also incorporated into the design. The combination of autonomy, extensive data acquisition, and design for long duration missions makes C-MASS a unique and valuable research tool. Table 1 compares C-MASS with other current related hardware.

Table 1 A comparison of C-MASS to related hardware for supporting microbiological experiments in space

	Autonomous	Visual imaging	1-g centrifuge	Variable fluid transfer	Carrier
CGBA		√			Shuttle
Biomodule	√	√			Shuttle, ELVs
Biorack		√	√		Shuttle
Biosample	√	√	√	√	Eureca
C-MASS	√	√	√	√	COMET, Shuttle, Freedom

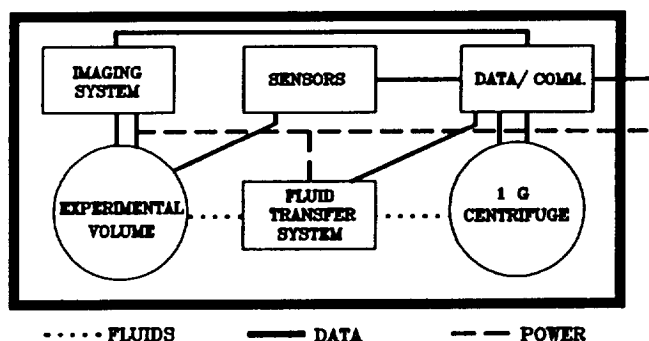


Fig. 1 A functional diagram of the C-MASS design

A functional diagram for C-MASS is shown in Figure 1. The diagram shows the six major subsystems and power, data, and fluid interfaces. Biological experiments are housed in the experimental volume and 1-g control centrifuge. The fluid transfer system is responsible for sample taking, nutrient delivery, waste removal, and organism transfer within the biological experiment vials. Data acquisition occurs through imaging systems and sensors. Imaging systems include photography, microscopy, and video for observing visible cellular changes in the microgravity of space. Sensors include spectroscopy and Enzyme-Linked ImmunoSorbent Assay (ELISA) for specific analytical techniques and environmental sensors such as vibration, radiation, temperature, pH, and light levels to record environmental conditions within the payload. The C-MASS communications system allows for data and video downlink as well as uplink, including in-flight

reprogrammability for adapting experiments while in progress. C-MASS is a small, low-cost, reliable payload designed to help fill the gap in space life science knowledge and technology.

C-MASS Subsystem Designs

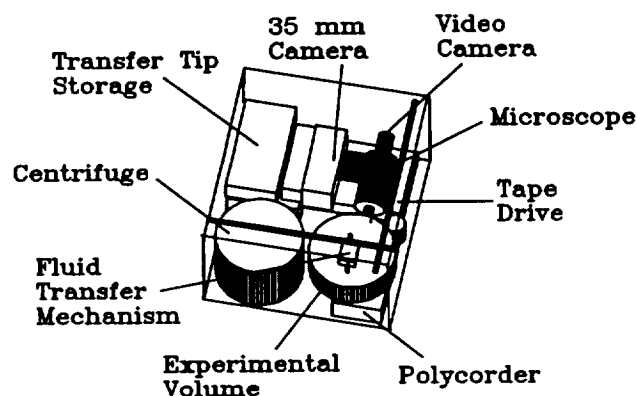


Fig. 2 Isometric view of C-MASS

An overall view of the C-MASS payload and its various subsystems is shown in Figure 2. C-MASS has outer dimensions of 11" x 14.5" x 15.75" for the limiting volume case of COMET. The design of the subsystems utilized an iterative process in which system requirements and objectives were identified. Next, design options using

current technology and off-the-shelf hardware, modified where necessary, were conceived for each system. Finally, trade studies were performed to determine the preferred method. The use of current technology and readily available hardware maximizes performance and reliability, while keeping costs and payload development time to a minimum. The subsystem descriptions that follow are reflective of this design philosophy.

Experimental Volume

All microgravity experiments will take place in the experimental volume; therefore, it must be capable of supporting various microorganism and cell culture experiments for up to 30 days. This entails allowing fluid transfers between individual experiment containers; providing lighting for photosynthetic organism growth, spectroscopy, and visual imaging; and facilitating data acquisition. The experimental volume is designed to maximize the total volume available for experimentation and data acquisition.

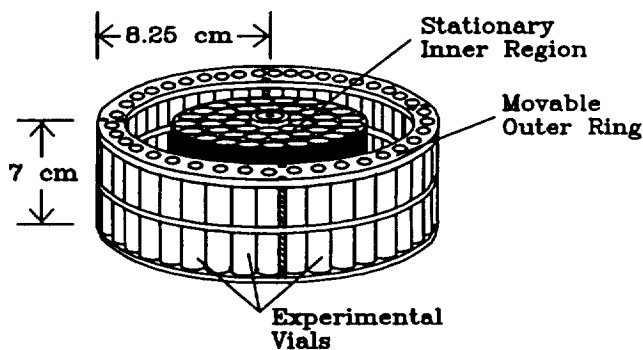


Fig. 3 Isometric view of the C-MASS experimental volume

The experimental volume consists of two separate regions: an outer positionable ring that permits visual data acquisition from experiments through the imaging system and an inner non-moving region. As shown in Figure 3, the entire volume is 8.25 cm in radius and 7.0 cm in height. The outer ring contains 40 individual experiment vials, each 1.2 cm in outer diameter and 5.0

cm high, for an internal fluid volume of approximately 3.0 ml per vial. These containers are placed with their centers at a radius of 7.6 cm from the center of the experimental volume with their long axis parallel to each other. The vial size and arrangement maximizes the number of experiments that may be conducted and exposed to the onboard data acquisition systems, while minimizing volume usage.

The space between the two regions of the experimental volume houses the various lighting sources used in C-MASS. An electroluminescent sheet provides ambient lighting for the outer experiment ring. A stationary light source is required for photography and microscopy, and several LEDs of varying wavelengths permit spectrophotometry as well as the use of fluorescent dyes and markers. The actual LED wavelengths can vary depending upon experimental requirements.

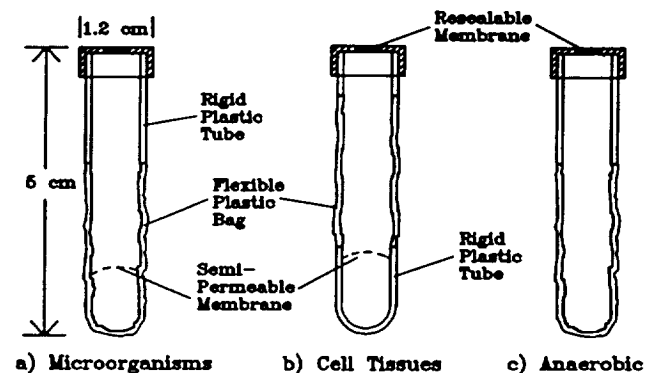


Fig. 4 Experimental vial options: a) microorganisms
b) cell tissues c) anaerobic bacteria

In order to maximize the experimental capabilities of C-MASS, a flexible experimental vial design was chosen. Three basic vial designs were conceived: the aerobic microorganism vial, the cell tissue vial, and the anaerobic vial. Figure 4 shows the containers all have the same basic components, simply organized differently. The four basic components are a rubber cap containing a reusable membrane; a clear, rigid, optically clear, plastic cylinder; a flexible plastic bag; and a semi-permeable membrane. The resealable membrane may be pierced by a fluid transfer needle, allowing fluid addition or removal, while maintaining system closure. The rigid cylindrical portion

of the vial allows for imaging of the experiments, and the flexible portion permits the internal volume to fluctuate with the introduction and removal of fluids, thereby maintaining a "hard-filled" fluid environment. Depending upon the type of experimental vial, a semi-permeable membrane is placed in either the rigid or the collapsible portion, or it may be eliminated entirely. In the aerobic microorganism configuration, the membrane effectively separates the vial into two regions: an upper section that contains organisms in a nutrient solution and a lower section containing gases. In the cell tissue configuration, the membrane allows nutrient replenishment in the upper compartment while keeping cells isolated in the remaining portion. Membrane porosity is matched to these different requirements. In addition, the elimination of the membrane provides a single environment for the study of anaerobic organisms.

For aerobic bacteria and other microorganism experiments, the organisms are contained within the rigid upper region of a vial. This allows for an aliquot (a small sample) to be taken by the fluid transfer system and used to inoculate a new nutrient-filled vial once the population has reached its saturation point. This process can be repeated many times, allowing the researcher to study changes in behavior and structure over multiple organism generations. In contrast, for cell tissue experiments, the cells are placed on the other side of the semi-permeable membrane. This allows for fluid removal and nutrient replenishment without damage to the fragile tissues.

The non-rotating inner portion of the experimental volume can hold up to 42 sample vials. These are similar to the anaerobic experiment vial, but with a shorter rigid section, and may contain nutrients, fixative, or other experiment support fluids. This area may also be used for additional experiments not requiring any inflight assay capability.

The final element of the experimental volume is the DC stepper motor which positions the outer experiments for the various assay techniques. The motor is connected to the experimental volume via sprockets and a nylon chain. The volume will only be moved slowly a few times a day. These short durations and low accelerations were deemed to have minimal effect upon the microgravity experiments.

1-G Centrifuge

C-MASS's launch, orbital, and landing environments introduce many variables that are difficult to simulate in ground-based control experiments. Temperature profiles, vibrational levels, and the extreme launch and reentry loads can all play significant roles in organism development. This problem is compounded by the inherent variability of living organisms. The best results would be obtained by comparing organisms from the same origin that have been exposed to identical conditions with the exception of gravitational accelerations. Therefore a small 1-g centrifuge is provided onboard C-MASS. The inclusion of the centrifuge ensures that any observed alterations in organism structure, function, or behavior are due solely to spaceflight changes in gravity.

The centrifuge design presents some of the most difficult hardware challenges for C-MASS. All commercial centrifuges are designed for much higher rotational rates than required for producing accelerations of 1 g. Also, all other centrifuges designed for use in microgravity are either too large or unmodifiable to this particular configuration. Therefore, the C-MASS centrifuge is a unique instrument, but one that utilizes commercially available or readily producible components. In this way, it remains consistent with the drive to use only off-the-shelf hardware.

To maximize the commonality of the experiments in the experimental volume, the centrifuge utilizes an identical circular arrangement using the same types of sample vials. Like the experimental volume, only the outer ring will be capable of motion, while the center portion is fixed to minimize rotating mass. The 7.6-cm radial distance to the center of the outer ring experiments requires that the centrifuge rotate at 108.5 RPM. Motor control is achieved using a small DC gear motor that utilizes a feedback loop to control motor output to within 2 RPM. The gravity gradient across any single experiment is +/- 0.11 g. Therefore, all rotating experiments will see accelerations between 0.89 g and 1.11 g during centrifuge operation.

Several options for fluid transfer within the centrifuge were examined. The simplest, most reliable, and least mass intensive of these is to simply stop the centrifuge for brief periods. Fluid transfer can then be accomplished

with the same device and in an identical manner to that used with the experimental volume. Although stoppage of the centrifuge for fluid transfer could affect experiments, it was determined that any changes would remain insignificant if the stoppage were for only a few minutes a day.

The identification of specific vial locations within the centrifuge is accomplished through the use of small photoelectric sensor and a machined groove of continuously varying depth in the vase of the centrifuge platter. By measuring the depth of this groove when the centrifuge comes to rest, the sensor and accompanying software can determine the relative position of any vial in the centrifuge. Based upon this information, the fluid transfer device can move to the appropriate vial. The accuracy of this system is well established in electronic micrometers.

Fluid Transfer System

The fluid transfer system provides C-MASS with the capability of supporting a wide variety of experiments. This versatile system has the ability to remove or add fluids, such as fixative and nutrient media, to each of the experimental vials. Fluid transfer is necessary to sustain the experiments for the entire mission duration. For instance, the system can remove wastes and replenish nutrients, or it can inoculate a few cells in a new nutrient solution. These tasks are accomplished without contamination of the individual experimental vials.

In order to successfully transfer fluid, the experimental vials must be accessed without loss of closure. To accomplish this, a transfer tip, which is similar to a syringe, punctures the resealable membrane at the top of the experimental vial. Once the transfer tip has been inserted into the experimental vial, a combination of two valves and a pumping mechanism are used to force a maximum of 1 ml of fluid either into or out of the transfer tip. This fluid transfer scheme, known to be extremely reliable, is derived from an automatic, battery-powered pipettor typically found in laboratories.

The transfer tip design not only facilitates fluid transfer, it also prevents contamination of the experiments and reduces possible cell damage as well. A flexible plastic bag has been attached to the transfer tip needle on the inside of the transfer tip. This bag fills with fluid as it is

pumped from the experimental vial, blocking the liquid from entering the pumping mechanism, thus inhibiting the contamination of the pump. Also, 110 transfer tips are located inside C-MASS allowing for transfer tip exchange, avoiding cross contamination between experiments. To reduce cell damage, the fluid inlet into the transfer tip is located on the side of the needle tip instead of on the end. The fluid inlet can then be larger, reducing the shear forces experienced by the delicate cells.

The experimental vials in both the centrifuge and experimental volume are accessed using a robotic arm. An end effector on the robot arm holds the fluid transfer pumping mechanism and a transfer tip. To accomplish the fluid transfer system requirements, the robotics arm is capable of movement in three dimensions. Three stepper motors are used in conjunction with three orthogonal leadscrews. A diagram of the robotic arm can be seen in Figure 5. The robotic control mechanism uses the positioning system, discussed in the 1-g centrifuge section, to locate the different vials.

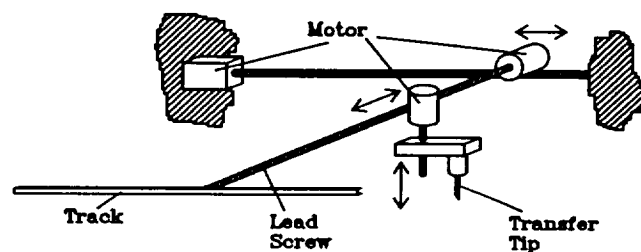


Fig. 5 Isometric view of the robotic fluid transfer arm

Imaging System

Organism growth and development is a dynamic, non-linear process. It is simply not possible to completely understand changes due to microgravity and their underlying mechanisms through the analysis of only an end result. Fixing experiments to preserve them for later ground-based analysis is often done, but it alters cell

structure and prevents their use for beginning new cell lines for terrestrial use. The loads experienced during landing can also alter or even destroy experiments. Therefore, the ongoing visual record provided by the imaging system is essential for establishing a time line of organism development. The imaging system is responsible for producing high quality visual data of the outer ring experiments in the experimental volume. The centrifuge experiments will not be imaged due to volume limitations. The imaging system is comprised of three major components: a microscope, a video camera, and a photographic camera. Also, samples saved for return can be compared to those studied *in situ* in the space environment.

Due to the size of the biological organisms under study within C-MASS, a microscope is required for all imaging applications. Basic light microscopy of unstained cells provides very poor resolution of cellular features, and provides only a two-dimensional view. This poor resolution may be greatly enhanced using techniques known as differential phase contrast and the Nomarski method (differential interference contrast). The C-MASS imaging system will utilize these techniques to view cellular features and organism surface texture. Objective lenses range in power from 10x to 1000x, providing proper magnification for a variety of imaging applications.

A video camera is included in the C-MASS design for its capability of recording organism motion and the ability to provide real time images for downlinking. The output from the microscope can be directed to the small charge coupled device (CCD) camera head. The camera has a resolution of approximately 400,000 pixels with a 12 bit color capability. The video output is stored for later retrieval on Earth. Video images can also be downlinked to a groundstation, permitting researchers a small real time glimpse of the activity within an experimental vial.

Photography has a far greater resolution than video imagery due to the much smaller size of light sensitive crystals in film as compared to a video pixel element. Therefore, a photographic camera is also provided within C-MASS to yield high resolution pictures of the experiments within the experimental volume. As with the video camera, imaging is through the microscope. The camera utilized is a commercially available 35 mm electronically controllable, auto focus, auto aperture, auto timing, auto film advance camera with a film back capable

of holding a 250-exposure roll of 35 mm film. Several options exist for increasing the number of possible exposures with simple modifications to various camera components. Modifications to the shutter and film advance mechanism would permit the use of multiple rolls of 110 film rather than a single 35 mm roll. The ability to section the film into smaller exposure sizes is also possible. The optimal method for maximizing photography capabilities may require combination of both modifications.

Sensors

The in-flight sensor measurements taken by C-MASS are extremely important since these will allow researchers to determine the dynamic effects of microgravity on experiments. Sensor selection was difficult because sensors placed in contact with organisms may form biofilms and condensate around them decreasing the accuracy of the sensor readings. Most of the sensors discussed in this section measure the environmental conditions in which the experiments take place. The sensors that fall into this category include temperature, illumination, gravity level, vibration, and radiation. C-MASS also incorporates a spectrophotometer, for obtaining experimental data concerning optical density, and ELISA for detecting the presence of specific biomolecules, such as proteins or peptides.

The wide variety of environmental conditions recorded are measured by the following sensors. The variations of temperature within C-MASS are measured using thermistors, small semiconductors that change their electrical resistance in response to temperature. These thermistors are strategically located throughout the facility and measure both air and surface temperatures between -30 and 100 degrees Celsius. Experiment pH is measured using one of the cameras in conjunction with an indicator chemical added to the solution. The pH indicator used is phenol red, or phenolsulfonphthalein, which changes from yellow to red between pH 6.4 and pH 8.2. Either the video camera or 35 mm camera can be used to photograph the vials containing the pH indicator. Video images can be downlinked for immediate quantification, or stored onboard until a later time. The illumination sensors are photoelectric diodes located in several different areas within the centrifuge and experimental volume. Accelerometers are used to measure both the gravity levels and vibrations between 40

micro-g and 10 g. Three orthogonal accelerometers are required to measure accelerations in three dimensions. Cumulative radiation is measured by three orthogonal film dosimeters, which are commonly used to detect radiation levels in laboratories.

The other sensors included in C-MASS provide analytical assay techniques. Spectrophotometry readings are obtained using several light sources and light detectors. The light sources are emitting diodes and the light detectors are photo diodes. There are six different light sources spanning the 200 mm to 1500 mm range, each aligned with a corresponding detector. Since the spectrophotometry sensors remain stationary, only the experiments located in the rotating part of the experimental volume have this assay capability. The other assay method is ELISA, a plastic sheet with specific antigens attached. These antigens are proteins that can chemically recognize other very specific reagent proteins. When the proteins come in contact with one another, the bound antigen produces a color change in proportion to the amount of reagent protein present. Using this method, very small amounts (as little as 10 picograms) of specific reagents can be detected and their concentrations determined. Currently, several hundred specific assays are possible using this technique.

Data/Communications

The valuable measurements taken by the sensors and imaging systems require a means of data storage and return. Communications are also necessary between Earth and C-MASS and between its various internal subsystems. The C-MASS is not only capable of downlinking data, but also of receiving uplink commands allowing for in-flight changes to be made. A standard XMODEM protocol is used for carrier-payload communications.

C-MASS provides the researcher with modular options in data storage. Depending on the amount of specified data to be taken, two different configurations can be used. In the event that large amounts of data are to be taken, an 8-mm magnetic tape drive will be placed onboard. Utilizing a modified tape changing mechanism, two 25-gigabyte tapes could be included for up to 50 gigabytes of storage for both sensor and video data. The second option is to store all sensor data in the 448K data acquisition scanner. This method would require that all

the sensor data be downlinked periodically instead of permanently recorded aboard C-MASS and that the video images be recorded on videotape. The advantage of the second option is that less volume is used which could allow, for instance, more fluid transfer tips to be available.

The data acquisition computer is the internal and external communications center for the payload. It controls the onboard operations of the subsystems such as sensors, fluid transfer, and environmental control. It also acts as the communications link with the ground receiving uplinked commands and downlinking requested data.

Cost Analysis

The total cost of construction and testing for C-MASS to produce a flight-qualified version of the payload was estimated at \$525,000. The actual hardware, modifications, and any raw materials made up only a small portion of that total, \$25,000, due to the predominant use of off-the-shelf components and commercially available products. Personnel costs for integration and testing made up the bulk of the estimate at \$500,000. This value covers the full-time salaries of an electrician, shop technician, project manager, and two other technicians for one year including overhead. It was presumed that this combination working in a small business setting could easily move C-MASS from its current preliminary design phase to ready-to use flight-qualified hardware within that time frame. The cost of C-MASS is competitive with other commercially developed space hardware of similar size and function, and it is much less than that of similar NASA-sponsored projects which take longer to complete.

C-MASS Science Experiments

Cells are the basic building blocks of life; therefore, an understanding of gravitational effects at the cellular level is absolutely necessary before large CELSS are created, astronaut health issues are treated, or the microgravity environment is used for commercial benefit. The specific physical processes altered in microgravity have both direct and indirect effects on cells which are still not completely understood. C-MASS, with its entourage of data acquisition hardware, can be used to document gravitational changes and alterations in ways not currently possible. C-MASS may also be used to take

advantage of the quiescent environment offered by microgravity for performing highly sensitive experiments not possible on Earth. In another way, the long-term exposure to reduced gravity offered by C-MASS will allow for experiments designed to reveal adaptations that organisms may undergo over long periods and many generations in a microgravity environment. The experiments C-MASS can support will help answer questions concerning how cells are affected by gravity, and they will provide a means to explore future commercial opportunities.

Gravitational cell biology research focuses on the response of a variety of physical phenomena to changes in gravity and the effect those changes have on life. The role of these physical phenomena in extracellular, intercellular, and intracellular processes determines the effect gravity has on cellular functions. Among these physical phenomena are sedimentation and convection. In the absence of gravity these processes do not occur. Other weak physical forces such as hydrostatic pressure and surface tension, normally dwarfed in the presence of gravity, become much more pronounced in the microgravity of space. The absence of sedimentation and convection combined with the enhancement of hydrostatic pressure and surface tension cause both internal (intracellular) and cell-to-cell (intercellular) changes in cell activities.

Gravity causes dense materials to settle or sediment at the bottom of a medium. Plant cells called statocytes use sedimentation to sense the orientation of the gravity vector. Starch granules in the statocyte fall to the cell bottom and react with the cell wall providing a directional reference for plant growth. Cells must also create cytoskeletal structures to inhibit the sedimentation of other organelles such as nuclei. Intercellular sedimentation affects the distribution of cells and materials. In the presence of gravity, prolonged contact between cells of different densities, or between cells and dense materials is impossible. For these reasons, in microgravity, cell differentiation unlike any observed in a terrestrial environment should occur.

Convection currents are caused when gravity acts on thermal and/or density gradients within a fluid. Intracellular convection is responsible for cytoplasmic streaming which transports signals and materials within a cell. Intercellular convection creates shear forces that

disturb cells and affect the way they develop and communicate with one another. These effects require better characterization, a job impossible to do in nominal gravity, to understand the mechanisms by which they act.

Hydrostatic pressure is responsible for the rise of fluid in a capillary tube, and surface tension is a measure of fluid adhesion forces. Hydrostatic pressure is important in examining the work done by a system ($W = P dV + V dP$). In the absence of gravity, the influence of these processes is much more pronounced, altering the fluidic environment both inside and outside cells. Therefore, as the pressure approaches zero, cellular events which involve a volume change, such as secretion or fission, are expected to be affected. Surface tension exists between a cell and its environment and between cells. Research in microgravity, where fluid can be easily manipulated, has provided insight into this behavior and should reveal the importance of fluid interactions in cellular functions.

The capabilities of C-MASS make it a valuable tool for supporting research that will identify and exploit the gravitational effects acting at the cellular level. The versatile fluid transfer system and variable experimental vials give C-MASS the ability to supply many types of organisms with different physical needs. The autonomous nature of C-MASS allows payloads on flights without the perturbations caused by crew presence that can ruin the quiescent microgravity environment.

The imaging systems and in-flight reprogrammability of C-MASS give investigators control over experiments from Earth to effect adjustments as needed during the flight. Onboard data acquisition from the sensors and spectroscopy will also provide a dynamic profile of experiments for postflight analysis which may help pinpoint key steps in cellular developmental processes. The environmental sensors will provide a time-dependent record of experimental conditions. This is crucial since cells and microbes are sensitive to variations in their surroundings. The data-intensive experiments made possible by C-MASS can be used to reveal how convection, sedimentation, hydrostatic pressure, and surface tension affect cellular processes.

Quiescent Environment Experiments

The absence of disturbing processes such as convection and sedimentation in microgravity make it an ideal

environment for performing delicate experiments on cells and biomolecules, the modification of cell lines, the growth of synthetic tissue cultures, and the polymerization of macromolecules may all benefit from experiments that take advantage of the quiescent environment of space. Genetically engineered cell lines have numerous commercial benefits which range from methods of drug delivery to the production of pharmaceuticals. Liposomes (lipid shells of vesicles) are presently used in the analysis of membrane proteins, therapeutic drug delivery, and in generating immunogenicity. Liposomes are extremely delicate structures. At 1 g, gravity-induced convection currents cause excessive fracturing of the bilayer resulting in small liposomes.⁴ A similar problem is encountered in cultured lymphocytes modified to destroy tumor cells in their host organism. Several billion cultured cells are needed per treatment and repeated treatments are necessary. However, only a few million viable cells are usually generated using processes on Earth. Lymphocyte cells grown *in vitro* suffer from fluid shear forces and poor nutrient and waste exchange causing low proliferation and misshapen cells.⁵ Both the liposome and lymphocyte experiments have shown increased growth size and an increase in lymphocyte production when performed in a microgravity or simulated microgravity environment. Therefore, further study is warranted in these areas, as well as a variety of other cellular studies which have only been carried out in 1 g.

Other experiments that may benefit from the quiescence of space are DNA recombination and molecular cloning. These processes are widely used by the pharmaceutical industry. The final product is material which can be used for the replacement or increased reproduction of any protein in the human body.⁶ It is possible that a microgravity environment would allow greater control over the production of human proteins in a host cell since it is known to affect both bacteria and liposome production. As in the two previous examples, a quiescent environment would minimize shear flow patterns caused by convection, guiding the cellular growth in a manner contrary to that found *in vivo*. It would also be possible to impose a small controlled force upon the cells as they grow. This may be especially useful in experiments such as the assembly of collagen, which has a well organized structure *in vivo*, but lacks this structure when growth is stimulated *in vitro*.⁷ Artificial development of well organized collagen has potential uses for surgical implants to replace damaged tissue.

Communication between cells is necessary for processes like differentiation where a cell uses the genetic material common to all cells to express its genes in a particular manner. For instance, one cell forms skin while another forms an eye, although both cells began with the same information. Complex intercellular communications and specific cytoplasmic elements appear to be the key factors governing differentiation. Data suggest that mammalian cells undergoing differentiation are more sensitive to gravitational effects than nondifferentiating cells and that changes in gene expression can be induced.⁸ One hypothesis presented by D. K. Kondepudi states that in microgravity a system evolving irreversibly toward an end status may proceed with equal probability to another end status, but a system that has evolved for generations at 1 g will give a single and well-known end.⁸ Cells communicate through chemical signals carried in a fluid intercellular space. A gravitational change in the fluid due to a reduction in particle streaming or convection will alter the cell's ability to transmit or receive signals from its surroundings. An understanding of these mechanisms could allow the control over the differentiation of one cell into a desired tissue type enabling transplantable organs to be grown when needed. The quiescent environment of space offers a laboratory where intercellular communication is altered and may be controlled more carefully than on Earth.

Electrofusion of plant cells to form hybrids is also enhanced in a microgravity environment due to the lack of sedimentation and convection.⁹ Plants are of interest for terrestrial uses such as pharmaceuticals and as food and oxygen sources for life supporting systems in space. The ability to hybridize a variety of plant cells permits scientists to breed plants with superior qualities such as greater biomass production with an increased resistance to disease. Ultimately, plants may be produced to support microgravity uses, lunar uses, and even Martian uses.

Multigenerational Experiments

In addition to a quiescent environment, COMET provides the opportunity to conduct experiments over a 30-day period which allows for the production of multiple generations of rapidly reproducing organisms such as bacteria. Bacteria are extremely well-studied and provide excellent building blocks for gravitational biology research. They are of interest not only for comparison to

Earth-based studies, but in anticipation for future space habitation. Microgravity experiments on the resistance of the bacteria *E. coli* to antibiotics¹⁰ and their metabolic adaptation¹¹ were performed previously on short duration flights. For reasons not yet known, some bacteria grow faster in microgravity and show an increased resistance to antibiotics. Other microorganisms such as the motile *Paramecium* are strongly affected by the gravity vector on Earth since they must expend energy swimming against it. In microgravity the proliferation rate of *Paramecium* increases, possibly because the energy previously used to move can be transferred to reproductive activities.¹⁰ However, to date no observed changes were maintained in subsequent generations on return to Earth. Multigenerational exposure to a reduced gravity environment may result in organisms genetically adapted to microgravity. Organisms which are unable to adapt will expire, and only those best suited for survival in microgravity will reproduce. Genetically adapted organisms returned to Earth may have specific traits that could be used in pharmaceutical production or for other scientific benefit.

The gravity-sensing properties of plants are currently under investigation, but a 30-day mission will allow observations of the development of some plants and photosynthetic cell colonies to maturity. One-celled algae, for instance, normally grow into an entire kelp plant on Earth. With its variable lighting capabilities, fluid transfer, and the ability to contain a variety of sample vial sizes, C-MASS could be used to observe the growth of a kelp colony under microgravity conditions. The results could provide valuable insights into gravity responses and cellular differentiation in space.

The multigenerational aspect of gravitational research is currently of interest as flights such as COMET become available and in anticipation for future long-term microgravity exposure on structures such as Space Station Freedom.

Experimental Protocol

Although the protocol for each experiment may differ, they all have similarities and can be adapted to the capabilities and constraints of C-MASS. The following is an example of a bacteria experimental protocol that is compatible with C-MASS.

One of a number of sample vials containing growth medium will be inoculated with organisms prior to launch. After microgravity is achieved, the next sample vial will be inoculated by the transfer of an aliquot from the first. Every subsequent 48 to 72 hours, when these samples have saturated the medium, an aliquot will be extracted and transferred to another vial containing fresh nutrients. The initial sample will then be fixed with gluteraldehyde for postflight analysis. The process will be repeated until the end of the mission, providing multiple generations of bacteria grown in space. Temperature, pH, radiation, and optical density will be measured, and visual imaging will be performed on each active sample during the flight. Specific products and metabolic markers will be visualized with ELISA strips throughout the mission. The experiment will provide a dynamic profile of long-term gravitational adaptations and changes in the bacteria studied.

Since C-MASS is already designed, built and tested, it is imagined that a team of National Institutes of Health (NIH) scientists and industrial scientists would simply deliver their respective samples to the launch site a few days prior to launch. These personnel would then monitor their experiments daily with data transmitted from the ground stations. And finally, they would receive postflight samples for more thorough analysis.

Conclusions

A preliminary design for C-MASS, a small payload capable of supporting a variety of microbiological experiments in space, has been presented. The important design characteristics have been described and examples of its capabilities and applications have been discussed. C-MASS adds a unique combination of autonomy, mission duration, and experimental capabilities not available with current space hardware. Furthermore, this design supports the low budget, high quality, and commercial application emphasis of SEI. It will provide researchers with generic hardware adding a needed component to the space infrastructure for biological research. Operationally, the researchers will use the C-MASS capabilities almost like another piece of laboratory equipment.

C-MASS may be used to satisfy scientific curiosity by offering a means to answer many of the basic questions concerning the effects of gravity on life. It may also be used to study cultured cells/tissues which are important in addressing astronaut health issues and the treatment of related diseases on Earth. In the future, C-MASS may be used to pave the way toward space commercialization by providing a facility that can utilize the space environment to produce novel biological products such as genetically altered species or specially modified cells. The important needs fulfilled by C-MASS and its commitment to SEI mandates make sit the next logical step in space life sciences development.

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